

## Totopotensamides, Polyketide–Cyclic Peptide Hybrids from a Mollusk-Associated Bacterium *Streptomyces* sp.

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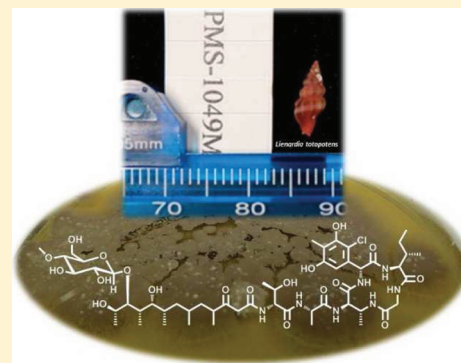
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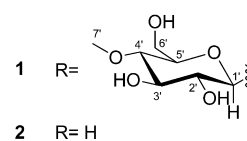
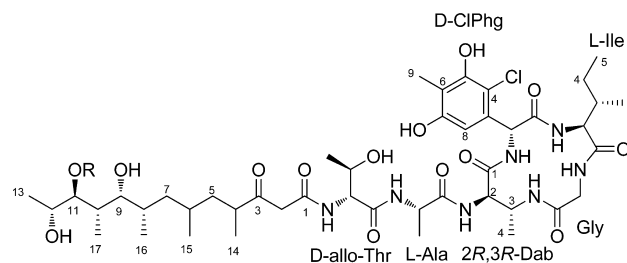
### S Supporting Information

**ABSTRACT:** Two new compounds, the peptide–polyketide glycoside totopotensamide A (1) and its aglycone totopotensamide B (2), were isolated from a *Streptomyces* sp. cultivated from the gastropod mollusk *Lienardia totopotens* collected in the Philippines. The compounds contain a previously undescribed polyketide component, a novel 2,3-diaminobutyric acid-containing macrolactam, and a new amino acid, 4-chloro-5,7-dihydroxy-6-methylphenylglycine. The application of Marfey's method to phenylglycine derivatives was explored using quantum mechanical calculations and NMR.



Microbial associates of marine animals provide an excellent and still comparatively untapped source of new molecules.<sup>1–3</sup> One hypothesis holds that examination of new animal groups will lead to the discovery of new animal–symbiont interactions and, therefore, to new chemistry. Shelled gastropod mollusks are relatively little explored for their symbiotic bacterial content, especially in terms of natural products discovery. We have been examining the natural products of bacteria cultivated from shelled gastropods as the source of new compounds with neuroactivity and other biological effects.<sup>4,5</sup> In particular, we have applied a collection methodology, *lumun–lumun*,<sup>6</sup> that has led to diverse and previously unappreciated mollusk taxa.<sup>6</sup> The method involves laying a net for a one- to six-month period, during which time the net is colonized by many different small mollusk species, some of which are as yet undescribed. In the course of these studies, a new species of conoidean mollusk, *Lienardia totopotens*, was found in a *lumun–lumun* net in the Philippines.<sup>7</sup> At the same time that the type specimen of this new species was collected, identical samples were obtained from the same net and used to cultivate bacterial associates, including *Streptomyces* sp. 1053U.I.1a.1b. Here, we report the characterization of structurally novel

peptides totopotensamides A (1) and B (2), isolated from *Streptomyces* sp. 1053U.I.1a.1b. Although the compounds did not exhibit biological activity in a wide range of assays, they contained relatively unusual structural features and so were further studied.



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Totopotensamide A (**1**) was isolated as a pale yellow solid and assigned the molecular formula  $C_{52}H_{84}ClN_7O_{19}$  on the basis of HRESIMS analysis. The isotopic distribution of the ions indicated the presence of chlorine. Analysis of the 2D NMR spectra, including COSY, HSQC, and HMBC in  $DMSO-d_6$  (Table 1 and Figure 1), allowed for the complete spectroscopic assignment of five common amino acids: one unit each of isoleucine, threonine, alanine, glycine, and 2,3-diaminobutyric acid (Dab). The  $^{13}C$  NMR spectrum (Table 1) showed six aromatic carbon signals representing five quaternary  $sp^2$  carbons ( $\delta_C$  131.3, C-3<sub>CIPhg</sub>; 111.8, C-4<sub>CIPhg</sub>; 153.2, C-5<sub>CIPhg</sub>; 114.5, C-6<sub>CIPhg</sub>; 155.8, C-7<sub>CIPhg</sub>) and one protonated  $sp^2$  carbon ( $\delta_C$  107.9, C-8<sub>CIPhg</sub>). The HMBC correlations (Figure 1) from the singlet methyl protons ( $\delta_H$  2.04, H-9<sub>CIPhg</sub>) to C-5<sub>CIPhg</sub>, C-6<sub>CIPhg</sub>, and C-7<sub>CIPhg</sub>, from the singlet aromatic proton ( $\delta_H$  6.61, H-8<sub>CIPhg</sub>) to C-3<sub>CIPhg</sub>, C-4<sub>CIPhg</sub>, and C-7<sub>CIPhg</sub>, and from an  $\alpha$ -proton ( $\delta_H$  5.71, H-2<sub>CIPhg</sub>) to C-3<sub>CIPhg</sub>, C-4<sub>CIPhg</sub>, C-8<sub>CIPhg</sub>, and a carbonyl carbon ( $\delta_C$  172.3, C-1<sub>CIPhg</sub>) together with their chemical shifts revealed the presence of 4-chloro-5,7-dihydroxy-6-methylphenylglycine (CIPhg). The amino acid sequence and cyclic structure of **1** were deduced from HMBC and NOESY correlations (Figure 1). HMBC correlations between the H- $\alpha$  protons or amide NH signals of one residue and the carbonyl carbons of adjacent residues indicated the sequence was Gly-Ile-CIPhg-Dab-Ala-Thr.  $^1H-^1H$  NOESY correlations between the H- $\alpha$  protons of one residue and the amide NH signals of adjacent residues (Figure 1) confirmed the sequence. The HMBC correlation from 3-NH<sub>Dab</sub> to the carbonyl and  $\alpha$ -carbons of Gly ( $\delta_C$  168.7; 43.7) established the Dab-Gly linkage and allowed assignment of the peptide macrocyclic portion of **1**.

By using the advanced Marfey's method,<sup>8,9</sup> it was possible to assign the absolute configuration of  $\alpha$ -carbons for the amino acid residues. Compound **1** was hydrolyzed, and the resulting amino acids were converted to both *N* $\alpha$ -(2,4-dinitro-5-fluorophenyl)-L-leucinamide (L-FDLA) and *N* $\alpha$ -(2,4-dinitro-5-fluorophenyl)-D-leucinamide (D-FDLA) derivatives, which were characterized by LC-MS in order of elution for D- and L-FDLA derivatives (Supporting Information). Thus, configurations were assigned as 2*R*-Thr, 2*S*-Ile, and L-Ala. For 2*R*-Thr, a large coupling constant between H-2<sub>Thr</sub> and H-3<sub>Thr</sub> ( $J_{H_2-H_3}$  = 12.0 Hz) and a NOESY correlation between H<sub>3-4</sub><sub>Thr</sub> and NH<sub>Thr</sub> revealed the absolute configuration of C-3<sub>Thr</sub> to be *R*.<sup>10</sup> Similarly, a large coupling between H-2<sub>Ile</sub> and H-3<sub>Ile</sub> ( $J_{H_2-H_3}$  = 10.0 Hz) in Ile and a NOESY correlation observed between H-6<sub>Ile</sub> and NH<sub>Ile</sub> revealed the absolute configuration of C-3<sub>Ile</sub> to be *S*. Using two authentic L-2,3-Dab (2*S*,3*R*-Dab and 2*S*,3*S*-Dab) standards, it was confirmed that the retention time for the L-FDLA-L-2,3-Dab derivative was earlier than the D-FDLA-L-2,3-Dab derivative (Supporting Information). The retention time for D-FDLA-D-2,3-Dab in **1** was identical to L-FDLA-2*S*,3*S*-Dab. So, D-2*R*,3*R*-Dab was identified in **1**. The 3*R* absolute configuration of 2,3-Dab in **1** was also confirmed by the small coupling constant between H-2<sub>Dab</sub> and H-3<sub>Dab</sub> ( $J_{H_2-H_3}$  = 3.5 Hz) and by a NOESY correlation between H<sub>3-4</sub><sub>Dab</sub> and NH<sub>Dab</sub>.<sup>10</sup> Furthermore, the acid hydrolysates of **1** were converted to *N* $\alpha$ -(2,4-dinitro-5-fluorophenyl)-L-alaninamide derivatives (L-FDAA), which were characterized by LC-MS in comparison with authentic standards (D-allo-Thr, D-Thr, and L-Ile). The results confirmed that **1** contained D-allo-Thr and L-Ile.

Because the use of Marfey's method with phenylglycine derivatives has not been rigorously explored, we synthesized standards using both D-phenylglycine (Phg) and L-3,5-dihydroxyphenylglycine (DhPhg). While the FDLA derivatives

of Phg exhibited the expected elution order by HPLC, the DhPhg elution order was the opposite of what is expected in comparison to proteinogenic amino acids. We further investigated this phenomenon using molecular modeling with quantum mechanical energy analysis. It was found that the lowest energy conformations of Phg-FDLA derivatives conform to the expected shape adopted by proteinogenic amino acids. By contrast, a strong H-bond between the DhPhg phenolic OH and the leucyl residue of FDLA stabilized a different conformation. This conformational difference drastically changes the relative orientation of the Leu and Phg  $\alpha$ -protons in the low-energy conformations (Figure 2). The  $\alpha$ -protons of the Leu and Phg both oriented to the same side of the dinitrobenzene plane in the *S,R* isomer, but opposite in the *S,S* isomer. By contrast, with DhPhg, there was less of an apparent difference in the orientation of the  $\alpha$ -protons of the Leu and DhPhg. Gratifyingly, when  $^1H$  NMR spectra of both diastereomers of Phg-FDLA and DhPhg-FDLA were compared, this prediction was borne out: there was a smaller difference in the  $\Delta\delta$  (0.24 and 0.08 ppm, respectively) of  $\alpha$ -protons of the Leu and DhPhg in the DhPhg-FDLA derivatives, while in Phg derivatives these values were 0.35 and 0.13 ppm, respectively (Supporting Information), supporting the reliability of the model. Quantum mechanical calculations indicated that CIPhg found in **1** should adopt a similar low-energy conformation to DhPhg (Figure 2). Because elution order is proposed to be based upon the shape adopted by derivatives,<sup>8</sup> and CIPhg and DhPhg should adopt the same shape, the configuration of CIPhg in **1** was assigned as D (*R*). Thus, amino acid configurations in **1** were determined to be L-Ile, D-CIPhg, 2*R*,3*R*-Dab, L-Ala, and D-allo-Thr.

The polyketide component of **1** was deduced as follows. A proton network from H-4 through H<sub>3</sub>-13 and four doublet methyls (H<sub>3</sub>-14, H<sub>3</sub>-15, H<sub>3</sub>-16, and H<sub>3</sub>-17) indicated a methyl-branched polyketide chain. HMBC correlations between C-3 ( $\delta_C$  210.4) and H-2, H-4, and H-5 indicated the presence of a ketone. Further, an isolated methylene spin system ( $\delta_H$  3.52 d (15.2), 3.42 d (15.2);  $\delta_C$  50.5, C-2) correlated by HMBC to a carbonyl at both C-1 ( $\delta_C$  167.5) and C-3. This placement predicted that the H-2 protons should be exchangeable. Indeed, incubation of **1** in CD<sub>3</sub>OD led to solvent exchange of the H-2 protons (Supporting Information). HMBC correlations from the NH ( $\delta_H$  6.24) of D-allo-Thr to polyketide C-1 established the connection between the polyketide and peptide portions of the molecule. The relative configuration of the C-8 to C-13 region was established by analysis of  $^1H-^1H$  coupling constants and NOESY data (Figure 3). C-11/C-12 was assigned as *anti* based on the small  $J_{H-11-H-12}$  value (2.6 Hz) and NOESY correlations of H-11/H<sub>3</sub>-13 and H-10/H<sub>3</sub>-13. C-10/C-11 was assigned as *anti* because of the large  $J_{H-10-H-11}$  value (7.8 Hz) and NOESY correlations of H-11/H<sub>3</sub>-17 and H-12/H<sub>3</sub>-17. The C-9/C-10 portion was *syn* based on the small  $J_{H-9-H-10}$  value (0 Hz) and NOESY correlations of H-9/H<sub>3</sub>-17 and H-8/H<sub>3</sub>-11. The C-8/C-9 portion was assigned as *syn* from the large  $J_{H-8-H-9}$  value (9.0 Hz) and NOESY correlations for H-9/H<sub>3</sub>-16 and H-7/H-10. The configurations of C-4 and C-6 could not be unambiguously determined.

Finally, the remaining NMR signals were assigned to a methyl-hexose moiety using  $^1H-^1H$  COSY and HMBC experiments (Figure 1). An HMBC correlation between H<sub>3</sub>-7' ( $\delta_H$  3.41) and C-4' ( $\delta_C$  80.8) determined placement of the methyl group, while an HMBC correlation from H-1' ( $\delta_H$  4.21) to polyketide C-11 ( $\delta_C$  88.4) determined the point of attachment to the polyketide chain. The relative configuration of the 4-methylhexose

Table 1. NMR Spectroscopic Data (500 MHz for  $^1\text{H}$ , 125 Hz for  $^{13}\text{C}$ ,  $\text{DMSO-}d_6$ ) for Totopotensamides A and B (1 and 2)

unit	no.	1		2	
		$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)
L-Gly	1	168.7, C		169.3, C	
	2	43.7, CH <sub>2</sub>	4.07, m; 3.44, m	43.8, CH <sub>2</sub>	4.07, m; 3.42, m
	NH		8.07, m		8.05, m
L-Ile $J_{\text{H2-H3}} = 10.0$ Hz in CD <sub>3</sub> OD	1	172.0, C		171.6, C	
	2	61.5, CH	3.77, m	61.9, CH	3.76, m
	3	35.6, CH	1.77, m	35.7, CH	1.75, m
	4	27.1, CH <sub>2</sub>	1.19, m; 1.05, m	27.1, CH <sub>2</sub>	1.23, m; 1.04, m
	5	11.5, CH <sub>3</sub>	0.78, t (6.3)	11.6, CH <sub>3</sub>	0.79, t (6.3)
	6	17.3, CH <sub>3</sub>	0.73, d (7.0)	17.2, CH <sub>3</sub>	0.74, d (7.0)
L-ClPhg	NH		7.09, d (6.0)		7.09, d (6.0)
	1	172.3, C		172.1, C	
	2	57.7, CH	5.71, d (10.0)	57.7, CH	5.71 d (10.0)
	3	131.3, C		131.2, C	
	4	111.8, C		112.0, C	
	5	153.2, C		153.3, C	
	6	114.5, C		114.6, C	
	7	155.8, C		155.8, C	
	8	107.9, CH	6.61, s	107.1, CH	6.61, s
9	11.3, CH <sub>3</sub>	2.04, s	11.0, CH <sub>3</sub>	2.03, s	
2R,3S-Dab $J_{\text{H2-H3}} = 3.5$ Hz in CD <sub>3</sub> OD	NH		8.93, d (10.0)		8.90, d (10.1)
	1	169.9, C		169.8, C	
	2	56.4, CH	4.46, m	56.5, CH	4.45, m
	3	46.7, CH	4.16, m	47.1, CH	4.09, m
	4	16.5, CH <sub>3</sub>	1.06, d (6.7)	16.6, CH <sub>3</sub>	1.06, d (6.5)
L-Ala	2-NH		8.02, d (8.2)		8.01, d (8.1)
	3-NH		6.24, d (9.7)		6.22, d (9.8)
	1	172.9, C		173.0, C	
D-allo-Thr $J_{\text{H2-H3}} = 12.0$ Hz in CD <sub>3</sub> OD	2	49.6, CH	4.35, m	49.7, CH	4.34, m
	3	19.6, CH <sub>3</sub>	1.19, d (7.0)	19.8, CH <sub>3</sub>	1.18, d (7.0)
	NH		8.05, d (8.0)		8.03, d (8.0)
	1	170.9, C		171.0, C	
polyketide	2	59.8, CH	4.23, m	60.1, CH	4.21, m
	3	68.4, CH	3.80, m	68.6, CH	3.80, m
	4	21.1, CH <sub>3</sub>	1.07, d (6.7)	21.1, CH <sub>3</sub>	1.07, d (6.6)
	NH		8.24, d (8.1)		8.24, d (8.1)
	1	167.5, C		167.6, C	
methyl-glucose	2	50.5, CH <sub>2</sub>	3.52, d (15.2); 3.42, d (15.2)	50.6, CH <sub>2</sub>	3.53, d (15.1); 3.41, d (15.1)
	3	210.4, C		210.4, C	
	4	44.8, CH	2.74, m	44.8, CH	2.72, m
	5	40.4, CH <sub>2</sub>	1.73, m; 0.77, m	40.4, CH <sub>2</sub>	1.72, m; 0.77, m
	6	29.2, CH	1.38, m	29.1, CH	1.38, m
	7	42.6, CH <sub>2</sub>	1.12, m; 0.78, m	42.6, CH <sub>2</sub>	1.10, m; 0.76, m
	8	34.9, CH	1.50, m	34.9, CH	1.51, m
	9	73.9, CH	3.53, m	75.5, CH	3.44, m
	10	37.6, CH	1.75, m	37.7, CH	1.78, m
	11	88.4, CH	3.41, m	79.8, CH	3.19, m
	12	67.6, CH	3.61, m	68.3, CH	3.54, m
	13	20.3, CH <sub>3</sub>	1.04, d (6.0)	20.2, CH <sub>3</sub>	1.06, d (6.1)
	14	19.1, CH <sub>3</sub>	0.99, d (6.8)	19.1, CH <sub>3</sub>	0.99, d (6.8)
	15	22.6, CH <sub>3</sub>	0.81, d (6.4)	22.6, CH <sub>3</sub>	0.80, d (6.5)
	16	17.9, CH <sub>3</sub>	0.84, d (6.4)	17.9, CH <sub>3</sub>	0.82, d (6.4)
	17	17.3, CH <sub>3</sub>	0.73, d (6.9)	17.3, CH <sub>3</sub>	0.75, d (6.9)
	methyl-glucose	1'	104.8, CH	4.21, d (8.2)	
2'		75.3, CH	2.99, dd (9.0, 8.2)		
3'		77.9, CH	3.29, dd (9.0, 8.6)		
4'		80.8, CH	2.91, dd (9.0, 8.6)		
5'		76.9, CH	3.14, m		
6'		62.1, CH <sub>2</sub>	3.60, m, 3.46, m		
7'-OMe		61.3, CH <sub>3</sub>	3.41, s		

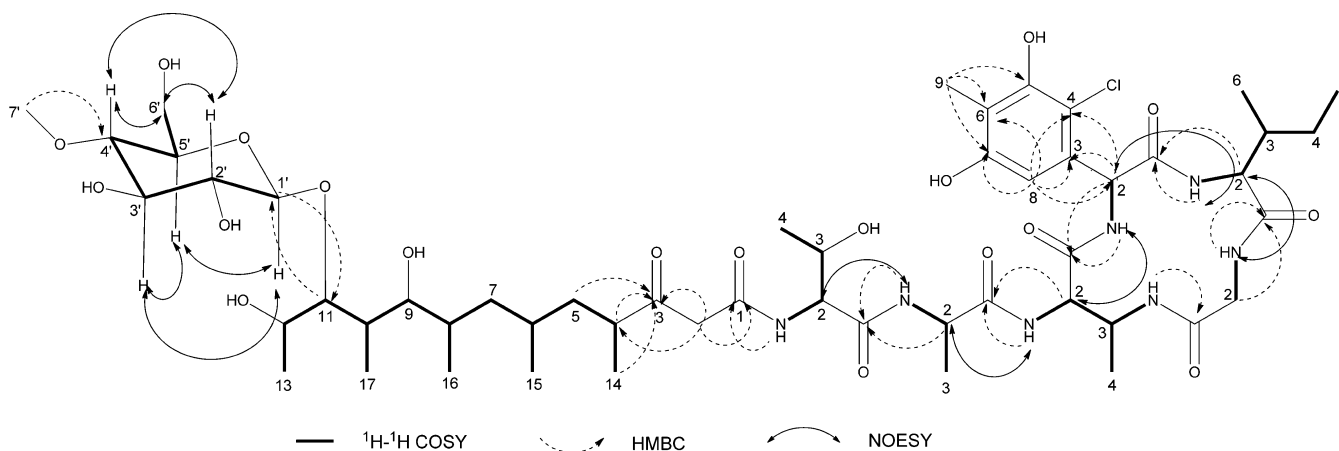


Figure 1. Selected  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC, and NOESY correlations for **1**.

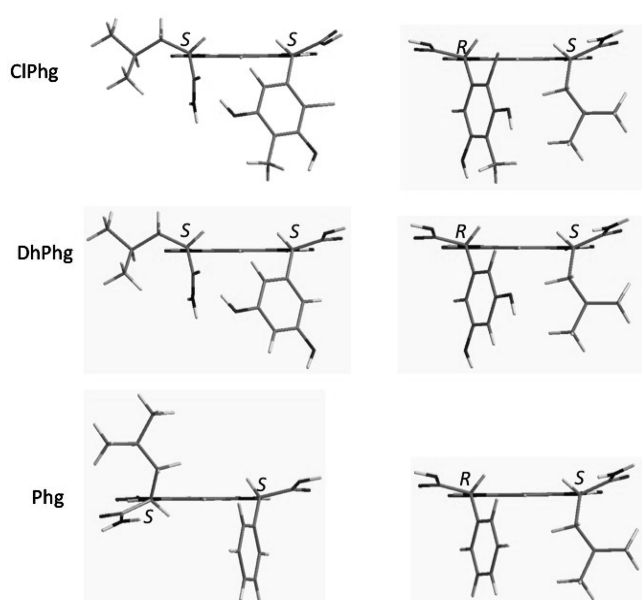


Figure 2. Molecular modeling of FDLA derivatives of Phg, DhPhg, and ClPhg with the lowest quantum mechanical energy.

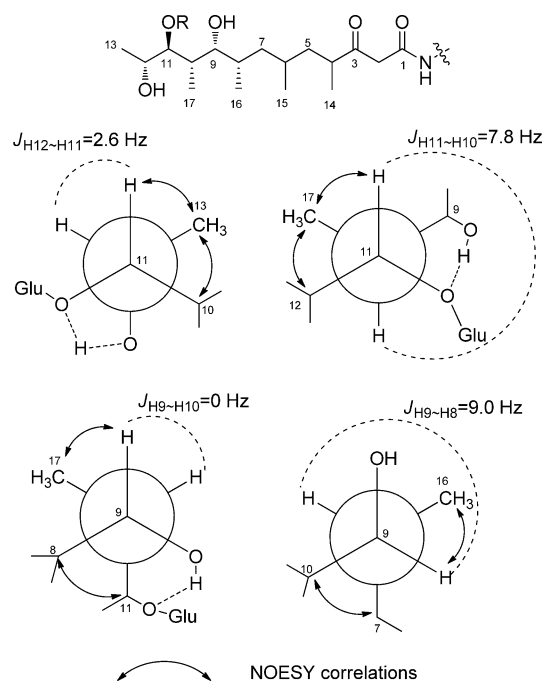


Figure 3. Key NOESY correlations and rotational model for the polyketide unit in totopotensamide **A** (**1**).

moiety was elucidated on the basis of NOESY and  $^1\text{H}$ - $^1\text{H}$  coupling constant data (Figure 1 and Table 1). NOESY correlations between H-1'/H-3'/H-5' suggested that the hexose moiety took a chair form with axial orientations for these protons. The *anti* orientations of these to H2' and H-4' were deduced from the large vicinal coupling constants ( $J_{\text{H-1}'-\text{H-2}'} = 8.2$  Hz,  $J_{\text{H-2}'-\text{H-3}'} = 9.0$  Hz,  $J_{\text{H-3}'-\text{H-4}'} = 8.6$  Hz,  $J_{\text{H-4}'-\text{H-5}'} = 9.0$  Hz). Thus, the 4-*O*-methylhexose moiety was assigned as 4-*O*-methyl-1- $\alpha$ -glucoside.

Totopotensamide **B** (**2**) was obtained as a pale yellow solid. Its molecular formula was determined as  $\text{C}_{45}\text{H}_{72}\text{ClN}_7\text{O}_{14}$  on the basis of HRESIMS data, which differed from **1** by the loss of  $\text{C}_7\text{H}_{12}\text{O}_5$ . Because of this mass difference and the absence of NMR signals for the methyl glucose unit (Table 1), **2** was assigned as the aglycone of **1**. Indeed, the NMR spectra were very similar, with the notable exception of an 8.6 ppm upfield shift of C-11 in **2** compared to **1**, supporting the absence of the glycosidic bond at C-11 in **2**. COSY, HSQC, and HMBC experiments further supported these assignments (Supporting Information). The absolute configuration of the components of

**2** were identical to those of **1**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, including coupling constants (Table 1), were similar in both compounds. The hydrolysate of **2** was analyzed using the advanced Marfey's method,<sup>8,9</sup> confirming that the absolute configurations of the amino acids in **2** were identical to those of **1**.

Totopotensamides are not closely related to known compounds and exhibit several unusual features not found in other natural products, to the best of our knowledge. They contain a new amino acid, ClPhg. Modified phenylglycine (Phg) is found occasionally in bacterial natural products, most notably in vancomycin-like compounds of the actaplanin<sup>11</sup>/ristomycin group.<sup>12</sup> The 2-position of the aromatic ring in Phg is relatively easily halogenated. For example, in risocetin this position was synthetically iodinated.<sup>12</sup> Although Dab is relatively common in nonribosomal peptides, the presence of Dab in such a small, constrained macrocycle has not been previously observed in natural products. Finally, the polyketide side chain has no

precedent, in that the organization of substituents such as methyl, hydroxy, and keto groups has not been previously observed. The functionality is somewhat related to macrolides, such as erythromycin and relatives,<sup>13</sup> but overall there are no reported compounds that are close to this organization. In addition, the  $\beta$ -ketoamide-amino acid motif is reminiscent of many tetramic acids.<sup>14</sup> In the case of tetramic acid synthesis, the amino acid undergoes a Dieckmann condensation with the side chain, leading to termination of chain extension,<sup>15</sup> but here the compounds are further extended by amino acids. Finally, 4-*O*-methylglucose is usually associated with eukaryotic metabolism, but it has been found occasionally in actinomycete products such as glycosylated nucleosides (tuberlicidin, dapiramicin).<sup>16</sup>

Totopotensamides were examined in a range of broad-net phenotypic assays, at concentrations up to 100  $\mu$ M. The compounds did not exhibit activity against panels of bacterial, fungal, or mammalian cell lines. They exhibited no effects on receptors or ion channels, as gauged using mouse dorsal root ganglion neurons and human receptor screens at the NIMH Psychoactive Drug Screening Program. The compounds caused no observable phenotypic effects on zebrafish larvae, nor did they affect the producing bacteria as hormones or in any other observable way. Thus, the biological activity of these unusual products if any has yet to be determined.

The discovery of new molecules from a bacterium associated with a new species of mollusk is perhaps only coincidence, but we feel that linking exploration for biodiversity and natural products can accelerate discovery in both areas. Complications in making this assessment include the difficulty in determining whether bacteria are truly symbiotic or whether they are casual associates of the animals in question. For example, although we have shown that actinobacteria are likely specific symbionts of cone snails,<sup>17</sup> in this particular case we do not know whether the cultivated bacteria are symbionts or casual associates. The *lumen-lumen* method, in providing access to animal diversity, has already been shown to yield new animal species and new animal venoms<sup>6</sup> and could also provide a rich source of new molecules from associated symbiotic bacteria. This report represents an early-stage exploration of that potential.

## ■ EXPERIMENTAL SECTION

**General Experimental Procedures.** Optical rotations were measured on a Jasco DIP-370 polarimeter. UV spectra were obtained using a Perkin-Elmer Lambda2 UV/vis spectrometer. NMR data were collected using either a Varian INOVA 500 (<sup>1</sup>H 500 MHz, <sup>13</sup>C 125 MHz) NMR spectrometer with a 3 mm Nalorac MDBG probe or a Varian INOVA 600 (<sup>1</sup>H 600 MHz, <sup>13</sup>C 150 MHz) NMR spectrometer equipped with a 5 mm <sup>1</sup>H[<sup>13</sup>C,<sup>15</sup>N] triple resonance cold probe with a z-axis gradient, utilizing residual solvent signals for referencing. High-resolution mass spectra (HRMS) were obtained using a Bruker (Billerica, MA, USA) APEXII FTICR mass spectrometer equipped with an actively shielded 9.4 T superconducting magnet (Magnex Scientific Ltd.), an external Bruker APOLLO ESI source, and a Synrad 50W CO<sub>2</sub> CW laser. Supelco Discover HS (4.6  $\times$  150 mm) and semipreparative (10  $\times$  150 mm) C<sub>18</sub> (5  $\mu$ m) columns were used for analytical and semipreparative HPLC, respectively, as conducted on a Hitachi Elite Lachrom System equipped with a diode array L-2455 detector.

**Bacterial Material.** *Streptomyces* sp. 1053U.I.1a.1b was cultivated from *Lienardia totopotens* obtained by professional collectors near Mactan Island, Cebu, Philippines, as previously described.<sup>5</sup> Relevant permission from local and national authorities in the Philippines was obtained prior to beginning this study. The strain was cultured from dissected hepatopancreas tissue and purified, and later the strain was recovered from a glycerol stock and used for further chemical analysis.<sup>5</sup>

The 16S gene was cloned using primers 8–27f (5'-AGAGTTT-GATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGT-TACGA CTT-3') and submitted to GenBank, accession number JN982561. The taxonomically described relative with the closest sequence match was *Streptomyces carnosus*.

**Fermentation and Extraction.** During the neurological activity screening by DRG assay<sup>5</sup> and chemistry screening by LS-DAD-MS, the extract of *Streptomyces* sp. 1053U.I.1a.1b was not active, but was shown to contain compounds with unusual MS ion features. In order to characterize the structures of those compounds, the strain was cultured at 30 °C with a Bioflo110 fermentor containing 10 L of the medium ISP2 (0.4% yeast extract, 1% malt extract, 0.4% glucose, 2% NaCl). After 8 days, the broth was centrifuged and the supernatant was extracted with HP-20 resin for 4 h. The resin was filtered through cheesecloth and washed with H<sub>2</sub>O to remove salts. The filtered resin was eluted with MeOH to yield an extract.

**Purification.** The extract (500 mg) was separated into four fractions (Fr1–Fr4) on a C<sub>18</sub> column using step-gradient elution of MeOH in H<sub>2</sub>O (40%, 60%, 70%, 80%). Fr2, eluting in 60% MeOH, was passed through a LH-20 column (to remove diketopiperazines). The early elution fractions from the LH-20 column were combined according to the HPLC analysis and further purified by C<sub>18</sub> HPLC using 35% CH<sub>3</sub>CN in H<sub>2</sub>O with 0.1% TFA to obtain compounds **1** (3.5 mg, *t*<sub>R</sub> = 6.4 min) and **2** (1.0 mg, *t*<sub>R</sub> = 7.7 min).

**Totopotensamide A (1):** pale yellow solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +20 (c 0.1, MeOH); UV (MeOH)  $\lambda$ <sub>max</sub> (log  $\epsilon$ ) 235 (2.11), 282 (1.45) nm; <sup>1</sup>H and <sup>13</sup>C NMR (see Table 1); HRESIMS *m/z* 1146.5598 [M + H]<sup>+</sup> (calcd for C<sub>52</sub>H<sub>84</sub>ClN<sub>7</sub>O<sub>19</sub>, 1146.5589).

**Totopotensamide B (2):** pale yellow solid; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +43 (c 0.1, MeOH); UV (MeOH)  $\lambda$ <sub>max</sub> (log  $\epsilon$ ) 235 (2.10), 282 (1.35) nm; <sup>1</sup>H and <sup>13</sup>C NMR (see Table 1); HRESIMS *m/z* 992.4793 [M + Na]<sup>+</sup> (calcd for C<sub>45</sub>H<sub>72</sub>ClN<sub>7</sub>O<sub>14</sub>, 992.4723).

**Acid Hydrolysis of Peptides.** Compounds **1** and **2** (100  $\mu$ g each) were separately dissolved in 6 N HCl (500  $\mu$ L) and heated in sealed ampule vials at 110 °C for 16 h. The solvent was removed *in vacuo*.

**LC-MS Analysis of D/L-FDLA Derivatives.** The acid hydrolysates of **1** and **2** were dissolved in H<sub>2</sub>O separately. To a 50  $\mu$ L aliquot of each were added 1 N NaHCO<sub>3</sub> (20  $\mu$ L) and 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide (1% solution in acetone, 100  $\mu$ L), and the mixture was heated to 40 °C for 50 min. The solutions were cooled to room temperature, neutralized with 1 N HCl (20  $\mu$ L), and then dried *in vacuo*. The residues were dissolved in 1:1 CH<sub>3</sub>CN–H<sub>2</sub>O and then analyzed by LC-MS. The analysis of the L- and D-FDLA derivatives was performed on a Supelcosil LC-18 column (150  $\times$  4.6 mm, 5  $\mu$ m) employing a linear gradient of from 25% to 70% CH<sub>3</sub>CN in 0.01 M formic acid at 0.5 mL/min over 45 min. The retention times of the D- and L-FDLA derivatives, respectively, were as follows: L-Ala: 29.00, 25.30 min, *m/z* 382 [M – H]<sup>–</sup>; D-allo-Thr: 21.29, 23.02 min, *m/z* 412 [M – H]<sup>–</sup>; D-Dab: 38.95, 44.35 min, *m/z* 705 [M – H]<sup>–</sup>; L-CIPhg: 39.22, 31.18 min, *m/z* 524 [M – H]<sup>–</sup>; L-Ile: 39.24, 31.16 min, *m/z* 424 [M – H]<sup>–</sup>.

**LC-MS Analysis of L-FDAA Derivatives.** L-FDAA was used to derivatize the acid hydrolysates of **1** and **2** and three standard amino acids (D-allo-Thr, D-Thr, and L-Ile). The reaction with L-FDAA was performed with the same procedure as that used for FDLA. The retention times of the L-FDAA derivatives were as follows: D-allo-Thr: 13.50 min, *m/z* 370 [M – H]<sup>–</sup>; D-Thr: 14.80 min, *m/z* 370 [M – H]<sup>–</sup>; L-Ile: 24.32 min, *m/z* 382 [M – H]<sup>–</sup>.

**Quantum Mechanical Energy Analysis of FDLA Derivatives of Phg, DhPhg, and ClPhg.** The conformational energetics of each isomer (L-Phg-L-FDLA, D-Phg-L-FDLA, L-DhPhg-L-FDLA, D-DhPhg-L-FDLA, L-ClPhg-L-FDLA, and D-ClPhg-L-FDLA) were studied using quantum mechanical energy analysis. To understand which conformation was energetically preferred, L-Phg-L-FDLA, D-Phg-L-FDLA, L-DhPhg-L-FDLA, and D-DhPhg-L-FDLA were each built in five likely starting conformations. An additional hydrogen-bonding conformation was also built for L-DhPhg-L-FDLA and D-DhPhg-L-FDLA. These starting structures were optimized with Gaussian09 using density functional theory.<sup>18</sup> Specifically, the PBEPBE exchange and correlation functional<sup>19,20</sup> was employed with the 6-311G++(3df,3pd) basis set

using automatic density fitting. To mimic the presence of water, the IEFPCM implicit solvent was used during optimization.<sup>21</sup> Four conformations each of L-ClPhg-L-FDLA and D-ClPhg-L-FDLA were also built and optimized, starting with the two lowest energy structures from L-DhPhg-L-FDLA and D-DhPhg-L-FDLA and adding the chlorine atom oriented either inward or outward.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

NMR data and HRESIMS data for totopotensamides A and B (1 and 2). Ion chromatograms of the D/L-FDLA derivatives and the L-FDLA derivatives of the hydrolysis product of totopotensamides. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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